THE JOURNAL OF INVESTIGATIVE DERMATOLOGY 67:327–332, 1976
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Vol. 67, No. 3
Printed in U.S.A.

IN VIVO STUDIES OF THE PATHOGENESIS OF COLD URTICARIA, CHOLINERGIC URTICARIA, AND VIBRATION-INDUCED SWELLING

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The disorders generally classified as physical urticarias include cold urticaria, local and generalized (cholinergic) heat urticaria, dermographism, pressure urticaria, and vibration-induced angioedema. These diseases have the common property of being reproducibly induced by physical stimuli and are not associated with an increased incidence of allergic disorders such as hay fever or extrinsic asthma in which offending allergens are identifiable. Studies of these diseases have involved attempts to identify circulating antibodies that might be responsible for the observed sensitivity or to determine the release of vasoactive principles in response to the inciting stimulus. In this paper we will summarize studies of mediator release and the mechanism by which such release occurs in cold urticaria, cholinergic urticaria, and vibration-induced angioedema.

COLD URTICARIA

Cold urticaria is characterized by the rapid development of hives and swelling after exposure to a cold stimulus. It can occur in any age group, has no predilection for either sex, and is not associated with other allergic disorders. The typical patient may become symptomatic while walking outside in the winter; however, pruritus and swelling are often most prominent when the person comes indoors and begins to warm. Only exposed portions of the body such as the face and hands are affected. Although cold foods such as ice cream may lead to swelling of the lips or tongue, pharyngeal or laryngeal edema are exceedingly rare and gastrointestinal symptoms are not seen. However, generalized exposure, for example while swimming, can lead to such massive mediator release that hypotension, fainting, and drownings can result [1].

A simple diagnostic test which reproduces this phenomenon is to place an ice cube on the patient's forearm for 5 min and then wait 10 min for any reaction to occur. Although everyone has evidence of redness after the ice cube is removed, this normally subsides during the next 5 min. The patient with cold urticaria usually complains of pruritus in the area between 2 and 4 min after removal of the ice cube and develops a large hive the size of the ice cube within 10 min. Figure 1 shows the result of a positive ice cube test demonstrating swelling which encompasses the area where the ice cube was placed as well as the area where ice water dripped down the patient's arm.

In order to identify the mediators released in cold urticaria, patients were studied in the following manner. An intravenous catheter was placed in the brachial vein and 5% dextrose in water was slowly infused. Baseline samples were obtained and the intravenous line was flushed with normal saline containing 15 U/ml heparin in order to prevent local coagulation. The hand was then lowered into a bucket of ice water (0°C) and submerged to about 2 inches above the wrist for 4 min. The hand was removed; a second sample was drawn; and subsequent samples were obtained after 2, 5, 8, 12, 15, and 20 min. A 5-ml sample was discarded immediately before each sample was collected in order to eliminate any intravenous fluid present in the catheter; the catheter was then flushed with heparinized saline. Samples for histamine and serotonin determinations were drawn into 12-ml plastic syringes containing 100 U heparin (0.1 ml of a 1,000 U/ml solution). Ten milliliters of blood were drawn and spun for 20 min at 900 *g*, and the plasma was separated with Pasteur pipettes. Samples for bradykinin determinations were collected into ethylenediamine tetraacetate (9 mg/10 ml blood) and hexadimethrine bromide (3.6 mg/10 ml blood).

Six patients with cold-induced urticaria were studied. Each had a positive ice cube test while tests for cryoglobulins, cold agglutinins, cryofibrinogen, and serology for syphilis were negative. The patients had a normal protein electrophoretic pattern and a normal serum IgE level. Samples were assayed for histamine by the radioenzymic procedure described by Beaven et al [2], while serotonin was determined by a double isotopic assay [3] based on the procedure of Saavadra et al. [4]. Bradykinin was assayed by radioimmunoassay [5].

The results of the assay for histamine release in 5 patients are shown in Figure 2. Within 10 min after the ice water challenge, a wave of histamine was obtained, the peak values ranging from 10 to 36 ng/ml. Patients complained of warmth and pruri-

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Abbreviations:
ECF-A: eosinophil chemotactic factor of anaphylaxis
PAF: Platelet activating factor
SRS-A: slow reacting substance of anaphylaxis

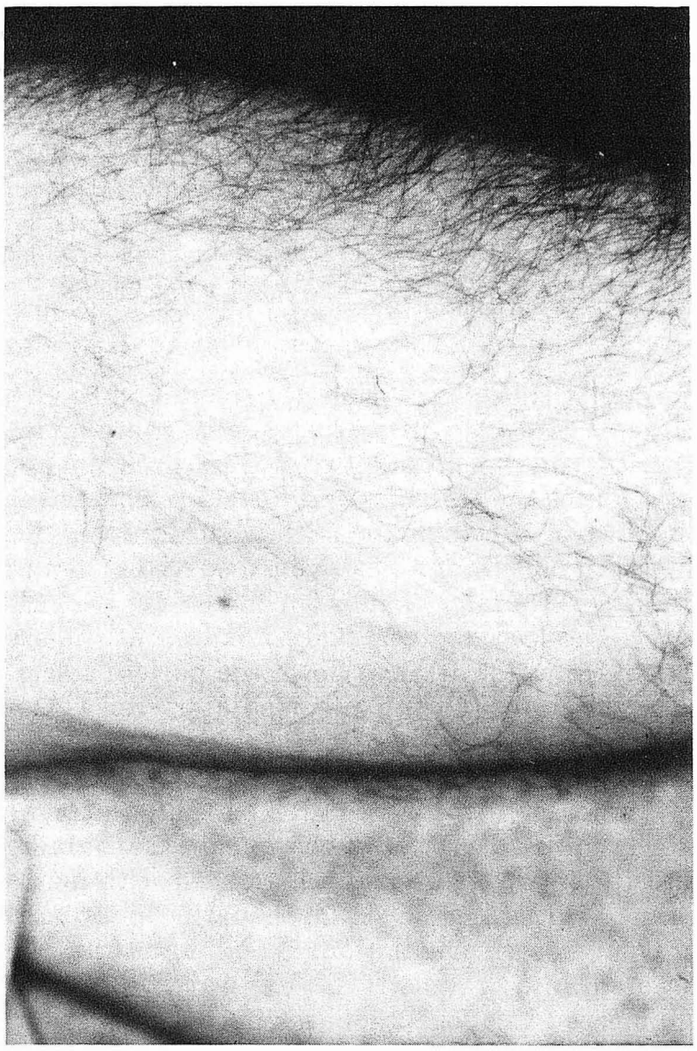

FIG. 1. Photograph of an ice cube test performed on a patient with cold urticaria.

tus at about 2 min after removing the hand from the ice water. Swelling was observed between 2 and 6 min, and peak histamine levels were obtained between 4 and 8 min. A single patient had a history of repeated episodes of hypotension caused by wading in the ocean up to his knees for a period of about 5 min. Such immersion caused flushing and light headedness that sometimes progressed to episodes of fainting. The assay for histamine release in this patient is shown in Figure 3. Four minutes after ice water challenge, the venous effluent from his hand contained 260 ng/ml histamine. Also shown are the blood pressure recordings obtained during this period; between 2 and 5 min after challenge the patient flushed, was mildly diaphoretic, complained of tightness in his chest, and had a transient drop in diastolic blood pressure of 50 points. Assays of the same samples in all 6 patients contained no detectable serotonin or bradykinin.

Passive transfer studies in cold urticaria were performed by injecting 0.1 ml of serum into each of two skin sites of the recipient and then challenging the sites with an ice cube 2 and 48 hr later as described above. All sera were prescreened to be sure they were Australia antigen-negative and free from bacterial contamination. Only 1 out of the 6 patients studied had a positive test at 2 hr and at 48 hr, suggesting an IgE-mediated reaction. This hypothesis was confirmed by passing the patient's serum over immunoadsorbents prepared with antisera against myeloma proteins of the IgG, IgM, IgA, IgD, and IgE classes of immunoglobulin. Only the anti-IgE immunoadsorbent removed the activity from the patient's serum.

CHOLINERGIC URTICARIA

Cholinergic urticaria or generalized heat urticaria is induced by an increase in body tempera-

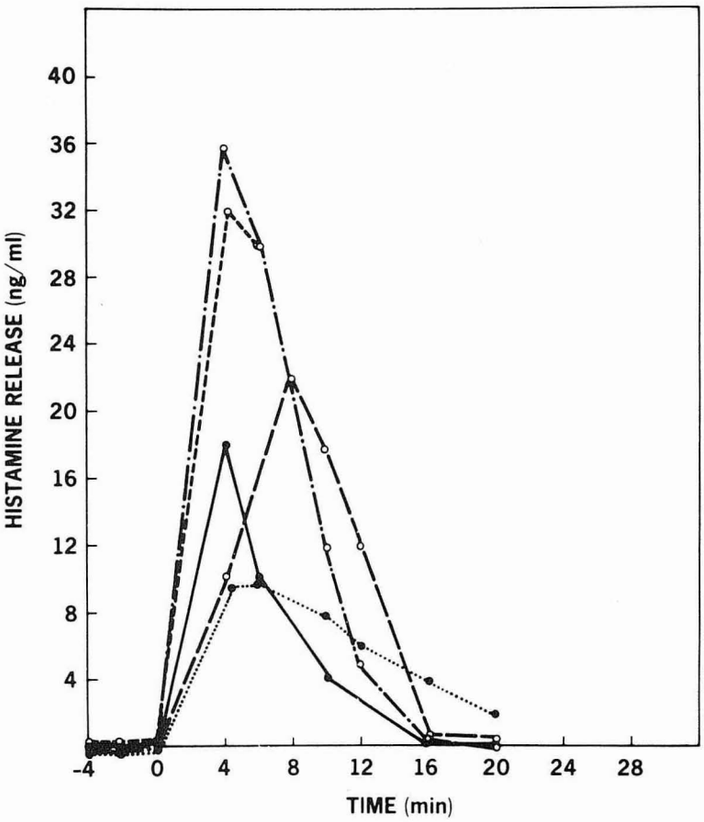

FIG. 2. Study of histamine release after ice water challenge in 6 patients with cold urticaria.

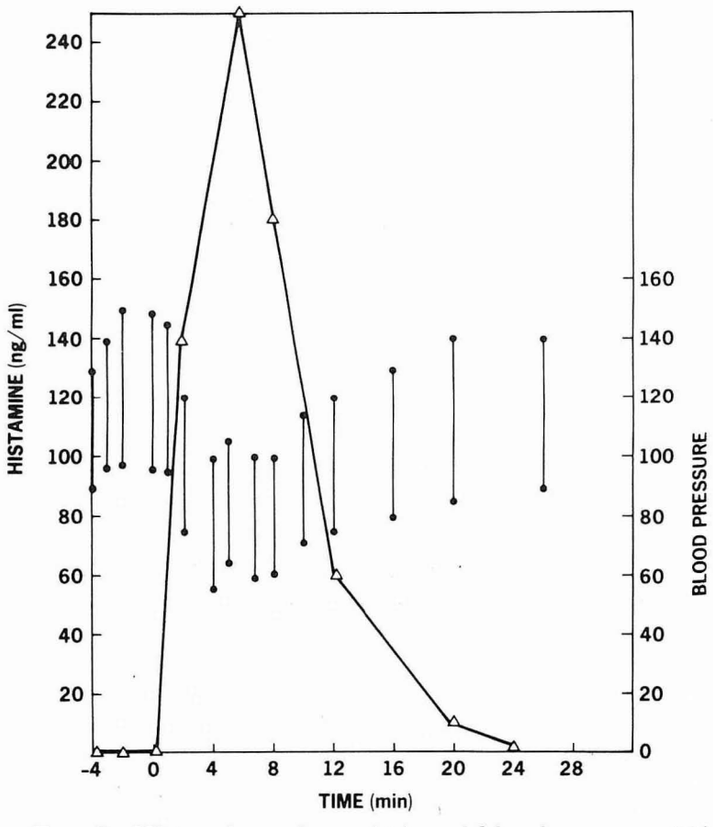

FIG. 3. Histamine release (△) and blood pressure (●) recording in a cold urticaria patient who had a history of cold-induced hypotension.

ture associated with exposure to a hot stimulus or as a consequence of exercise or psychic stimuli [6, 7]. Each of these challenges appears to initiate a neuroreflex which results in the elaboration of acetylcholine from parasympathetic and/or sympathetic neurons followed by pruritus and urticaria. The lesions are characterized by small punctate wheals surrounded by considerable erythema which tend to first appear over the upper thorax and neck. Patients appear to be "hypersensitive" to cholinergic stimuli since intradermal injection of mecholyl or acetylcholine produces a positive wheal and flare reaction often surrounded by smaller satellite lesions. This reactivity can be used as a test for cholinergic urticaria; it is diagnostic when satellite lesions are seen and is suggestive of the disorder if a wheal appears. Figure 4 summarizes the neuroreflex pathway thought to mediate cholinergic urticaria. Studies in support of such a scheme include the observation that if a tourniquet is placed on one arm of a patient and the hand immersed in warmed water, no local or systemic reaction is observed. However if the tourniquet is released, a systemic reaction results [8] suggesting that some central perception of the temperature change at the periphery is necessary. Since the tourniquet cannot interrupt afferent neurogenic stimuli it is assumed that perception of a temperature change in the venous effluent has occurred.

Patients with cholinergic urticaria were studied with the same intravenous catheter setup used for cold urticaria. As a challenge, patients were instructed to run in place for 10 min and samples were obtained every 5 min for 35 min. In Figure 5 is shown a photograph of the hives which had developed in a patient 10 min after completing the exercise test. We have thus far studied 6 patients with cholinergic urticaria. In a single patient with extremely severe symptoms, a clear-cut increase in plasma histamine was observed as shown in Figure 6. This patient had a positive mecholyl skin test with satellite lesions and, after exercise challenge, had markedly pruritic wheals over the entire body, many of which became confluent. After 5 min of exercise, an increase in plasma histamine was observed. It then rose abruptly to 25 ng/ml after 7 to 8 min and gradually declined over the next half hour. In 2 of 4 other patients with milder symptoms, a positive mecholyl skin test without satellite lesions was observed and a similar histamine release curve was obtained. However the peak levels reached were 3 ng/ml and 4 ng/ml, respectively, while their baseline values were less than 1 ng/ml. In the other 2 patients no elevation of plasma histamine was detected. The sixth patient, reported previously [9], had an atypical presentation because the lesions were not pruritic and no erythema was seen, but prominent wheals covered the entire body and the mecholyl skin test was

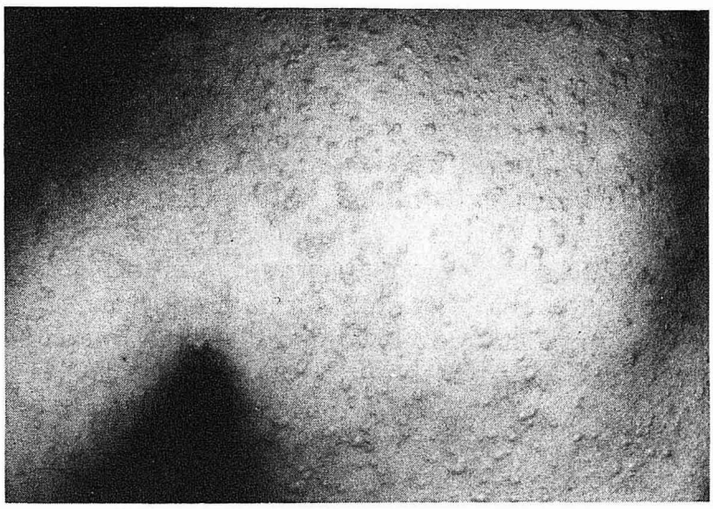

FIG. 5. Photograph of a patient with cholinergic urticaria 10 min after exercise, showing the characteristic small, punctate wheals.

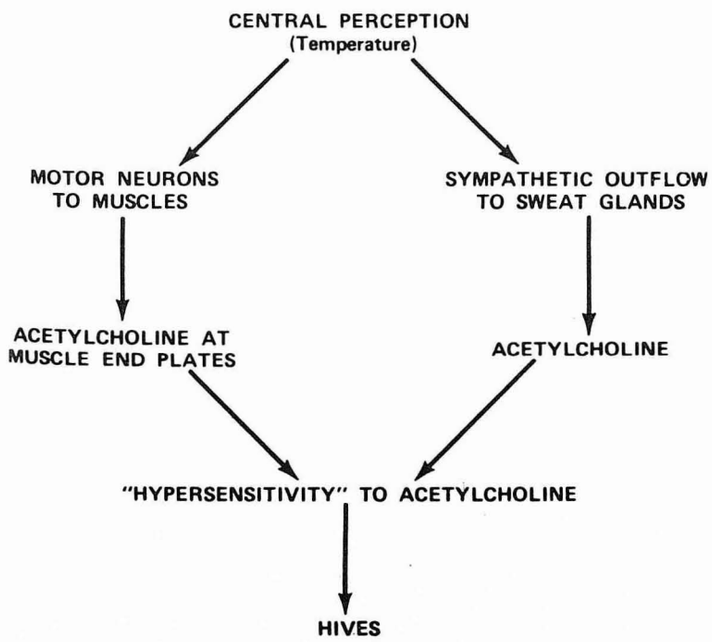

FIG. 4. Proposed neuroreflex mechanism leading to acetylcholine release in cholinergic urticaria.

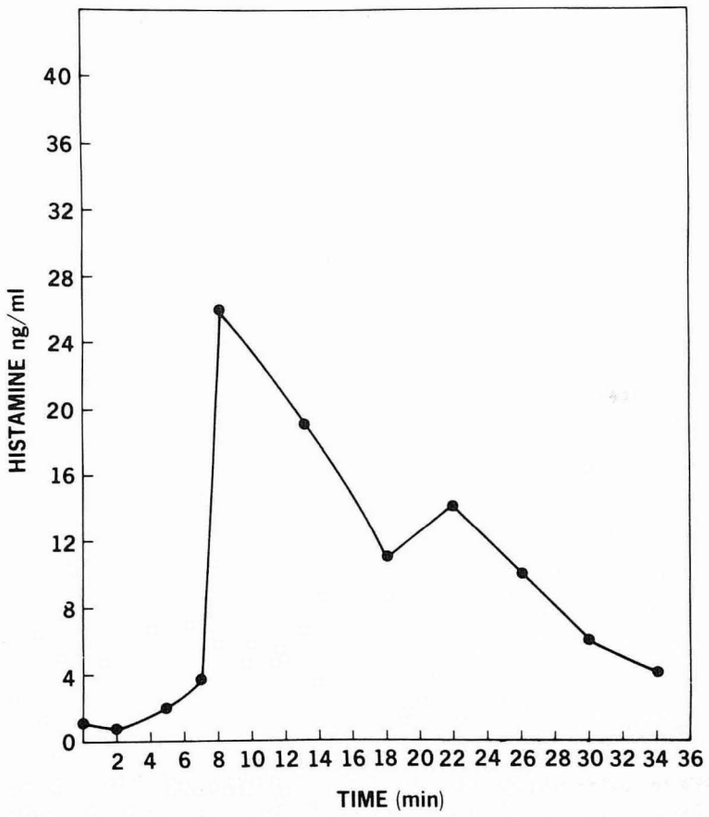

FIG. 6. Exercise-induced histamine release in a patient with cholinergic urticaria.

positive with numerous satellite lesions. This patient was studied twice and in each instance elevated baseline serotonin levels were obtained, which diminished, and then increased again during exercise. The time course of the serotonin values did not parallel the course of development of the patients' lesions. However, these repeatedly elevated values were the only abnormal serotonin determinations in any of our patients. No detectable elevation of bradykinin was found in any patient with cholinergic urticaria.

VIBRATION-INDUCED SWELLING

In 1972, a family was described by Patterson et al [10], who presented with angioedema induced by vibratory stimuli such as rapidly moving a towel back and forth across the back. In order to design a reproducible challenge in these patients, a laboratory vortex was set at a fixed speed, the patient's arm rested on a surface, and the forearm gently placed upon the vortex for 4 min. When we studied this patient [11] with a catheter in the brachial vein of the arm being stimulated, a rapid rise in plasma histamine was obtained within 1 min of application of the stimulus, reaching a peak value of 53 ng/ml. Within 3 min the patient's forearm and half the arm swelled circumferentially. The stimulus was removed after 4 min, and by this time the plasma histamine level had begun to return to baseline. Thus, this familial disorder yielded the most rapid rise and fall of plasma histamine in response to a physical stimulus that we have so far encountered.

When we tested patients with cold urticaria, cholinergic urticaria, idiopathic chronic urticaria, and normals for vibration-induced swelling, we found that many people responded with localized angioedema extending over a slightly greater area than was in contact with the stimulus. We did not observe any response which yielded rapid circumferential edema as did patients with the hereditary disorder. However, 2 patients with cholinergic urticaria responded with erythema, warmth, and swelling over most of the volar surface of the forearm. A facial flush could be reproducibly induced in 1 of these patients in response to this vibratory stimulus. We therefore studied both these patients in the manner described above. The results obtained in the patients and a normal control are shown in Figure 7. A rapid rise in plasma histamine was observed in the patients with a pattern identical to that observed in hereditary vibratory angioedema; however the magnitude of the response was considerably less. The normal control did not have an elevation in plasma histamine in response to the stimulus.

DISCUSSION

Studies of histamine release in cold urticaria have not previously yielded reproducible results. Elevation of whole blood histamine in such patients after cold challenge was first reported by Rose [12]. Subsequently Henderson et al [13] obtained similar results; however, no control group of patients was included. Duner et al [14] found the blood histamine levels elevated in both cold urticaria patients and normal controls. Plasma histamine determinations have similarly yielded conflicting results. Beall [15] reported elevated plasma histamine levels in a single cold urticaria patient after cold challenge, while Spuzic and Ivkovic (16) reported no change in plasma histamine levels in 2 cold urticaria patients. Utilizing the radioenzyme assay for histamine in heparinized plasma (or serum) we have been able to demonstrate elevated plasma histamine levels in all our cold urticaria patients. The time course of histamine release appeared to coincide with the onset of pruritus and swelling. By comparison we found the determination of histamine in whole plasma by bioassay or fluorometric assay to be less sensitive and to give poor reproducibility. We did not detect elevated levels of either serotonin or bradykinin in these patients and cannot support the observations of DeLaus and Winkelman [17] who suggested that kinins may be critical mediators of cold urticaria. In a single patient, approximately 10 times the maximum histamine level of any other patient was obtained at a time that significant hypotension was observed. We conclude that histamine release does occur in patients with cold urticaria as a consequence of cold challenge, and it appears likely that it is the major mediator contributing to the pruritus, swelling, and hypotension, characteristic of this disorder [9].

We found only a single patient in whom we could demonstrate an IgE-dependent mechanism by passive transfer, although an incidence as high as two-thirds has been previously reported by Houser et al [18]. Wanderer et al [19] reported 2 patients in whom IgM appeared to be of pathogenic importance; however, we could not demonstrate passive transfer of cold urticaria after either short (2–4 hr) or long (24–48 hr) latent periods in the 5 other patients. The mechanism by which a change in temperatuare could lead to mediator release is unknown although the critical cell would appear to be the tissue mast cell. Somehow this cell is

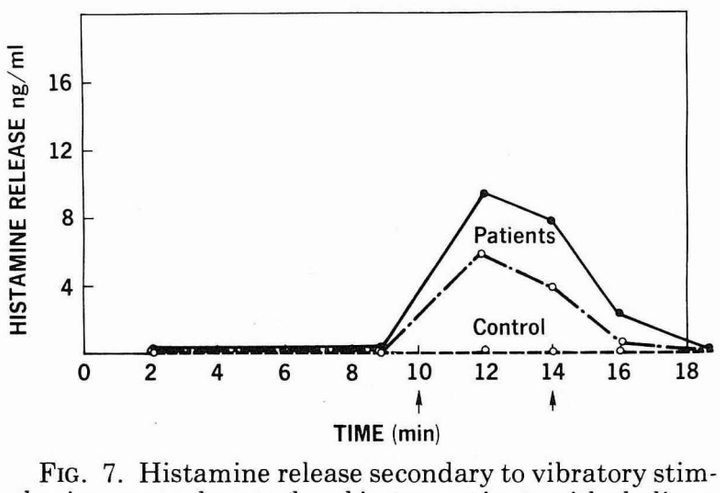

FIG. 7. Histamine release secondary to vibratory stimulus in a normal control and in two patients with cholinergic urticaria and vibration-induced swelling.

"sensitized" or activated by a drop in temperature followed by mediator release as the temperature increases. By analogy with the mechanism of mediator release in allergic rhinitis or extrinsic asthma it is likely that slow reacting substance of anaphylaxis (SRS-A) [20], the peptides designated eosinophil chemotactic factor of anaphylaxis (ECF-A) [21], and platelet activating factor (PAF) [27] are also released. However, we have not assayed for SRS-A or ECF-A and have obtained no evidence of PAF release since elevated plasma serotonin levels were not observed. We have recently reported that histamine itself is also selectively chemotactic for human eosinophils [23]; however, it is unlikely that the rapid release and clearing of the histamine observed could lead to local eosinophil accumulation.

Less is known about the pathogenesis of cholinergic urticaria. Although patients appear to be hypersensitive to cholinergic stimuli, and a neuroreflex mechanism appears to be operative, there is no evidence that the disease is immunoglobulin mediated and the mechanism by which cholinergic agents form a wheal is unknown. Our studies of mediator release in this disorder are not clear-cut. In some patients, no elevation of histamine, serotonin, or bradykinin was detected. In 2 patients, elevated histamine levels were observed which appeared to correlate with the patients' response to exercise; and in a single, atypical patient, elevated serotonin levels were obtained which did not correlate well with the patient's course. Technical limitations may contribute to the negative findings observed in some patients since the urticaria involves the superficial layers of skin rather than the deep dermis or subcutaneous tissue (which *are* affected in cold urticaria). Furthermore, sampling a brachial vein during exercise can only reflect the systemic circulating level at that moment rather than the level directly draining the site being stimulated. The results further suggest that this disorder may be heterogeneous since both the clinical presentation and results of mediator release have not been uniform.

Our evaluation of a patient with hereditary vibratory angioedema suggests some intrinsic defect of the patient's mast cells which leads to degranulation after the appropriate stimulus. The observation that a similar, although milder form of vibration-induced swelling is observed in some patients with cholinergic urticaria suggests that there may be a pathogenic relationship between the two disorders. They are nevertheless distinct clinical entities since none of these cholinergic urticaria patients complained of swelling induced by vibratory stimuli.

REFERENCES

1. Horton BT, Brown GE, Roth GM: JAMA 107:1263, 1936
2. Beaven MA, Jacobson S, Horakova Z: Clin Chim Acta 37:91, 1972
3. Beaven MA: *In* Handbook of Psychopharmacology, Vol I. Edited by L Iverson, SD Iverson, SH Snyder New York, Plenum 1975, p 253
4. Saavadra JM, Brownstein M, Axelrod J: J Pharmacol Exp Ther 186:508, 1973
5. Talamo RC, Haber E, Austen KF: J Lab Clin Med 74:816, 1969
6. Grant RT, Pearson RSB, Comeau WJ: Clin Sci 2:253, 1936
7. Hopkins JG, Kesten BM, Hazel OG: Arch Dermatol 38:679, 1936
8. Baer RL, Harber LC: *In* Immunological Diseases, vol II. Edited by M Samter. Boston, Little, Brown, 1971, p 973
9. Kaplan AP, Gray L, Shaff RE, Horakova A, Beaven MA: J Allergy Clin Immunol 55:394, 1975
10. Patterson R, Mellies CJ, Blankenship ML, Pruzansky JJ: J Allergy Clin Immunol 50:174, 1972
11. Metzger WS, Kaplan AP, Beaven MA, Irons J, Patterson R: J Allergy Clin Immunol (in press)
12. Rose B: J Allergy 12:327, 1941
13. Henderson LL, Code CF, Roth GM: J Allergy 29:122, 1958
14. Duner H, Pernow B, Sterky G: Acta Allergol (Kbh) 16:228, 1960
15. Beall GN: J Allergy 34:8, 1963
16. Spuzic I, Ivkovic L: Acta Allergol (Kbh) 16:228, 1961
17. DeLaus FV, Winkelmann RK: Arch Dermatol 98:67, 1968
18. Houser DD, Arbesman CE, Ito K, Wicher K: Am J Med 49:23, 1970
19. Wanderer AA, Maselli R, Ellis EF, Ishizaka K: J Allergy Clin Immunol 48:13, 1971
20. Ishizaka T, Ishizaka K, Orange RP, Austen KF: J Immunol 104:335, 1970
21. Kay AB, Austen KF: J Immunol 107:899, 1971
22. Benveniste S, Papoian R: Fed Proc (abstr) 31:748, 1972
23. Clark RAF, Gallin JI, Kaplan AP: J Exp Med 142: 1462, 1975

DISCUSSION

Provost: Have you been able to find specific antibody activity for the serum IgE in your cold urticaria patients? Do they have positive skin tests?

Kaplan: The patients in whom an IgE mechanism was demonstrable were not clinically atopic and the skin tests to our usual battery of antigens were negative, including ragweed.

Austen: How would physical stimuli initiate IgE-dependent reactions?

Kaplan: In cold uticaria there are many possibilities that might explain the observed mediator release. One has to rule out an IgE cryoglobulin but evidence against this is the fact that passive sensitization of basophils from patients who have shown to be "acceptors" for IgE with the patient's plasma or isolated IgE does not release histamine upon chilling and warming the cells. This suggests that since skin is responsive we may be dealing with a mast cell which releases mediators when the temperature changes after an abnormal IgE binds to it, or that the patient has an IgE antibody to a temperature-dependent skin antigen.

Jordon: Soter and others have recently suggested that some forms of urticaria may be manifestations of systemic vasculitis. In the patients with cholinergic urticaria without increased histamine levels, were any of these patients hypocomplementemic or was there any immunofluorescent evidence in skin lesions suggestive of vasculitis?

Kaplan: The patients I described have no complement abnormality, no change in serum complement with challenge, and immunofluorescent staining of their tissue after challenge reveals no evident complement deposition. Bear in mind that some patients with cold urticaria

do have cryoglobulins or other abnormal circulating proteins, and in these cases the pathogenesis of their lesions may involve other pathways of mediator release.

Sams: In another form of physical urticaria, namely solar urticaria, "reverse" passive transfer is possible; that is, exposure of the skin of the recipient to ultraviolet radiation followed by injection of the patient's serum will result in a hive. Mechanistically, this implies that ultraviolet releases one or more substances that may become antigenic in some individuals. Is this "reverse" passive transfer possible in cold urticaria?

Kaplan: We have not observed positive ice cube tests in cold urticaria if the serum is injected and the ice cube placed immediately thereafter (a minimum of 2–4 hr passive sensitization appeared necessary). Nor is a positive response seen if the site is chilled with an ice cube and the serum then injected.

Soter: The time-dependent release of ECF-A was demonstrated in the cold-challenged extremities with a peak between 2 and 5 min and a return to baseline by 20 min. The identity of the released eosinophil chemotactic activity with ECF-A was confirmed by chromatography on Sephadex G-10.

Kirkpatrick: Do eosinophils occur in the lesions of cold urticaria?

Soter: Eosinophils do not accumulate in tissue biopsy specimens from lesional sites examined during the first 30 min.

Kaplan: Eosinophils are not seen when cold urticaria lesions are biopsied. This is really not surprising because a concentration gradient of mediators is required over time in order to obtain a chemotactic response; the mediator release observed in cold urticaria is rapid and the mediators are rapidly cleared into the circulation so that there may be insufficient time for the stimulation of cell movement. Furthermore, the large quantities of histamine and ECF-A released into the tissue space would rapidly deactivate any eosinophils entering the periphery of the reaction and inhibit their further movement.

Kantor: Why don't antihistaminics work well in cold urticaria?

Kaplan: The drug of choice for cold urticaria is cyproheptadine (Periactin) which controls the patient's symptoms and turns the ice cube test negative. Diphenhydramine, a much better antihistamine, is not as effective. Cyproheptadine is said to have antiserotonin and antibradykinin activity, but we found no evidence of release of measurable amounts of these mediators. I would suggest that cyproheptadine has some other activity such as upon the release of mediators rather than simply antagonizing their effect that we do not appreciate. I do not think we can achieve a sufficient concentration of diphenhydramine at the relevant skin site to inhibit the action of histamine because the dose required would cause severe side effects.

Cohen: A still unexplained clinical observation is the reported superiority of one of the weakest antihistaminic preparations by in vitro standards, hydroxyzine, to suppress spontaneous and pressure-induced whealing in chronic urticaria of unknown etiology. Can your patients with clear-cut demonstrable histamine or cholinergic-related mechanisms possibly serve as models to help clarify this pharmacologic effect of hydroxyzine; have you had an opportunity to evaluate this preparation in either cold or cholinergic urticaria?

Kaplan: The efficacy of hydroxyzine in cholinergic urticaria may relate to inhibition of the neural reflex since it does have effects upon the central nervous system. This may be more important than its antihistamine activity and may also explain its effectiveness in relieving pruritus in urticaria of diverse etiologies.

Windhorst: Classical concepts of cholinergic urticaria emphasize that the pattern of disease and the cholinergic innervation of eccrine sweat glands suggest that the abnormal event is peculiar to this particular site of acetylcholine secretion. Have you found any evidence to suggest that this is or is not the case?

Kaplan: I did indicate that innervation of the sweat glands is by cholinergic fibers and that injection of cholinergic agents leads to the type of wheal and flare reaction seen in cholinergic urticaria. It is unknown whether this is a direct effect of the cholinergic stimulus or whether other mediators are recruited. I presented some evidence to support the latter alternative although an immunologic mechanism is not evident.

Austen: One might speculate that the mast cell mediators initiate the cholinergic skin reflex by analogy to the action of histamine on airway irritant receptors.

Weston: You mention that histamine itself is chemotactic for eosinophils. How does histamine compare in potency to other known chemotactic substances for eosinophils, such as ECF-A, C5a, and the Hageman factor-activated plasma chemotactants?

Kaplan: We have shown that histamine is indeed selectively chemotactic for eosinophils at concentrations between 10 and 300 ng/ml (peak at 120 ng/ml); this activity diminishes as the dose increases to 1000 ng/ml. This diminished response at high doses is reversed by H_2 receptor antagonists. As Dr. Austen noted, mast cells release a high-molecular-weight chemotactic factor which is not selective for eosinophils, peptides which preferentially attract eosinophils (ECF-A), and histamine. The net cellular response involves all of them; however, their qualitative effect upon the cells is not the same, and their relative quantitative contribution has not been assessed.

Rocklin: In view of the fact that there are two types of histamine receptors and that classic H_1-receptor antagonists are ineffective in the treatment of patients with cold urticaria, have you attempted to treat these patients with H_2-receptor blockers such as metiamide or burimamide?

Kaplan: We have not tried H_2 receptor antagonists in the treatment of cold urticaria. The classical wheal and flare response appears to involve the interaction of histamine with H_1 receptors.